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THE ASSAY, CHARACTERISTICS, COMPOSITION, AND ORIGIN OF OPIUM*

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The assay of morphine in opium, and determination of the total of minor phenolic alkaloids

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In continuation of the international programme of scientific research undertaken pursuant to resolutions 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General is presenting the following paper on the assay of morphine in opium, from the laboratory of the Narcotics Bureau of the Republic of China. The method of assay is designed to make it possible to determine the important non-phenolic alkaloids of opium on the same sample used for the morphine assay. In addition, it yields a figure for the percentage of the minor phenolic alkaloids, which will be of considerable interest to opium chemists.

* Inclusion of an article by contributing scientists in this series does not necessarily mean that the Secretariat shares the views expressed.

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THE ASSAY OF MORPHINE IN OPIUM, AND DETERMINATION OF THE TOTAL OF MINOR PHENOLIC ALKALOIDS

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Introduction

1. The purpose of this paper is to present an extraction method for the assay of morphine in opium, designed to provide for the possible determination of the important non-phenolic alkaloids on the same sample.

2. For this reason the opium is extracted with dilute acid, instead of lime water, and then the solution is made alkaline and the non-phenolic alkaloids are extracted with ether.

3. In preliminary work on the method, no dichloracetic acid was available to the Taiwan Narcotics Laboratory, and the attempt was made to remove minor phenolic alkaloids by two steps: filtration of the solution buffered to p H 6, and extraction of the acidic buffered solution with carbon tetrachloride. The latter step was carried out to remove porphyroxinemeconidine, and doubtless some similar alkaloids with it. However, it appears that not all the minor phenolic alkaloids were removed by these steps.

4. When dichloracetic acid became available to the Taiwan Laboratory, the method of separating the other phenolic alkaloids from morphine, essentially as given in the Secretariat's document ST/SOA/SER.K/12, was found of most value, and has been incorporated in the present method, in place of the two steps mentioned in paragraph 3.

5. Besides the assay for morphine content, and separation of the non-phenolic alkaloids, the method now yields a determination of the total of minor phenolic alkaloids not extractable by ether from alkaline solution. From the few results already obtained it appears that this figure is sometimes less than one per cent, but occasionally even above two per cent.

Summary of the assay

6. A weighed opium sample is extracted with dilute sulfuric acid in order to get all the alkaloids into solution.

7. An aliquot portion of the filtered acidic opium solution is rendered alkaline with sodium hydroxide, treated with a small amount of potassium sulfite, and extracted with ether, in order to remove non-phenolic alkaloids (principally papaverine, narcotine, thebaine, cryptopine, and codeine).

8. The alkaline solution is neutralized with sulfuric acid to litmus and concentrated to a small volume. A definite amount of dichloracetic acid is added and the solution extracted with chloroform in order to remove phenolic alkaloids other than morphine.

9. The acid solution is then rendered ammoniacal with ammonia water in excess and extracted with chloroform-alcohol mixture. Morphine is determined by titrating with standard sulfuric acid.

10. The purity of the assay morphine is then tested according to the method described in document ST/SOA/SER.K/12.

Experimental

A. The assay:

11. Weigh accurately 5 grammes of air-dried raw opium, triturate in a mortar with 5 cc of 0.1 N sulfuric acid to a smooth paste and then with 24.4 cc more of the same acid. Continue the trituration for half an hour in order to make a complete solution of all the opium alkaloids.

12. Filter the mixture and transfer with a pipet 20 cc of the filtrate, representing 2 grammes of dried opium, to a light-liquid continuous extraction apparatus. Add 3 cc of 3 N sodium hydroxide and 0.1 grammes of potassium sulfite, and extract with pure ether. After 3 hours of extraction, test the residue obtained by evaporating 1 cc of the ether taken from the side tube of the extraction apparatus for no reaction of codeine with Marquis' reagent.

13. When the extraction is complete, transfer the whole content of the extraction column to a separatory funnel (not including the ether extract in the receiving flask), with the necessary precaution of washing thoroughly the extraction column with small portions of water and adding to the separatory funnel. Separate the alkaline solution from the ether layer into a dish. The ether is then washed with 2 cc of 0.5 N sodium hydroxide and with 2 cc portions of water until the residue, obtained by evaporating 5 drops of the last washing, gives a negative reaction for morphine with Marquis' reagent. Add the washings to the main aqueous solution in the dish. Combine the washed ether with the main ether extract in the receiving flask and reserve the ether extract for the determination of papaverine, narcotine, thebaine, cryptopine, and codeine. (This determination will be described separately.)

14. Neutralize the alkaline solution in the dish with 1 N sulfuric acid to litmus and concentrate the mixture on the water-bath to about 10 cc.

15. Transfer the solution to separatory funnel No. 1, wash the dish thoroughly with small portions of water and add the washings to the separatory funnel. Add 0.4 cc dichloracetic acid and extract the solution with chloroform.

Transfer the $CHCl_3$ with shaking through the following solutions: in separatory No.2 - 7.5 cc water plus 0.1 cc dichloracetic acid; No.3 - 5 cc water; No.4 - 10 cc water plus 10 drops of NH_4OH; No.5 - 10 cc water. In transferring the $CHCl_3$ from separatory No.1 to No.2, filter through a pledget of cotton in a short-stemmed funnel. Finally filter the $CHCl_3$ from separatory No.5 through a pledget of cotton into a flask. Occasionally test the residue from a little of the $CHCl_3$ extract with Froehde's reagent (green, then violet). Continue the extractions with 20 cc portions of $CHCl_3$ until the $CHCl_3$ drawn from separatory No.5 gives no more reaction with Froehde's reagent. (Be sure that no morphine reaction appears).

16. Combine the acid solutions by pouring from the second separatory into the first, and from the third into the second and then into the first. Then put 5 cc water in the third separatory, shake and pour it into the second. Put again 5 cc water in the third separatory, make it ammoniacal with 1-2 drops of NH_4OH and shake, then pour it into the second. Finally add 5 cc water to the third separatory.

17. Add ammonia water to the first separatory funnel to render the aqueous solution basic to litmus with ammonia water, add 1 cc in excess, and extract with chloroform-alcohol mixture (80:20). Transfer the solvent into the second separatory, shake and transfer it to the third. Shake and transfer the solvent into a flask. Continue the extractions with portions of chloroform-alcohol mixture, until 5 drops of the last solvent extract after evaporating off gives no morphine reaction with Marquis' reagent.

18. Distil off the solvent and dry at 100° C for 15 minutes. Add from a buret a definite quantity of standard 0.1 N sulfuric acid into the flask and warm on the water-bath to ensure complete solution of the base. Titrate back with 0.1 N sodium hydroxide, using methyl red indicator. The number of cc of the 0.1 N acid consumed multiplied by 0.02853 gives the weight of morphine in 2 g of opium.

19. Distil off the chloroform from the extract of paragraph 15, and dissolve the residue in a few cc of 0.02 N sulfuric acid from a buret, add methyl red indicator, titrate back with 0.02 N sodium hydroxide, and calculate the minor phenolic alkaloids in terms of morphine.

Discussion

24. Dilute sulfuric acid is used for preparing the solution of total opium alkaloids instead of lime water, for the acid solution probably contains all the alkaloids of opium without any appreciable loss: thus a systematic determination for all the important alkaloids in opium will be made possible on one sample, whereas the lime solution dissolves quantitatively only codeine and some phenolic alkaloids, besides morphine. This is rather important in examining a small sample of opium as completely as possible.

25. The total volume of dilute sulfuric acid is made to total 29.4 cc. Taking into consideration the volume produced by the extractives in opium, which is taken as about 0.2 cc per gramme of opium, the total volume of the final acid solution is assumed to be 30 cc.

26. Ether is used to extract all narcotine, papaverine, thebaine, cryptopine, and codeine from the sodium hydroxide solution of opium with no loss of morphine, so that a complete determination of the above alkaloids can be made as part of the same procedure. The preliminary removal of these non-phenolic alkaloids, especially codeine, renders the subsequent extraction of the dichloracetic acid solution with $CHCl_3$ more complete, for codeine is far more difficult to remove than the minor phenolic alkaloids in this way.

27. Potassium sulfite is used to prevent the oxidation of morphine during the extraction of the alkaline solution with ether.

28. The separation of the minor phenolic alkaloids is carried out by the extraction of the opium solution containing dichloracetic acid with chloroform according to the pattern of the method given in document ST/SOA/SER.K/12.

29. The percentages found for minor phenolic alkaloids differ considerably with different samples, and are much higher than those reported for minor phenolic alkaloid in ST/SOA/SER.K/12. It should be noted that the method of the United Nations Secretariat described in the document cited yields only the value for minor phenolic alkaloid not extractable by chloroform from alkali, whereas the present method yeilds the total of minor phenolic alkaloids not extractable by ether from alkali. This probably explains the difference, since chloroform is known to extract some of the minor phenolic alkaloids from alkali rather readily; but it is also possible that the acid extraction of opium may yield a greater total of such alkaloids than the lime method.

B. Confirmation test for the purity of the assay morphine:

20. Transfer the titrated solution of morphine in the assay to a separatory funnel, wash the flask thoroughly with small portions of water, add the washings to the separatory, add 3 cc of 3 N sodium hydroxide and extract with ether. Test the extract for codeine, thebaine, papaverine, cryptopine, and narcotine with the different appropriate reagents.

21. Separate the aqueous solution from the ether, transferring it into a second separatory funnel. Wash the ether with 2 cc of water and add the washing to the aqueous solution in the second separatory funnel. Acidify the solution with 1 M phosphoric acid, extract with 2-3 portions of CCl_4 in order to remove the indicator.

22. Render the aqueous solution in the second separatory funnel basic to litmus with an excess of $NaHCO_3$ and extract with chloroform. Transfer the $CHCl_3$ with shaking through the following solutions: in separatory No. 3 - 10 cc water plus 0.4 cc dichloracetic acid; No. 4 - 7.5 cc water plus 0.1 cc dichloracetic acid; No. 5 - 5 cc water; No. 6 - 10 cc water plus 10 drops concentrated NH_4OH; No. 7 - 10 cc water. In transferring the $CHCl_3$ from separatory No. 3 to No. 4, filter it through a pledget of cotton in a short-stemmed funnel. Finally filter the $CHCl_3$ from separatory No. 7 through a pledget of cotton into a flask. Test the $CHCl_3$ extract with Froehde's reagent. If any non-morphine reaction is noted, continue the extractions with 20 cc portions of $CHCl_3$, and determine the minor phenolic alkaloids as in paragraph 19.

Some results

23. The following are a few results obtained by the Assay on some U. N. samples of opium:

	Opium sample	Assay		Confirmation test	
		Total phenolic minor alkaloids* (per cent)	Morphine (per cent)	Non-phenolic alkaloids in morphine	Other phenolic alkaloids in morphine
U.N.377	China, A	0.52	10.82	0	0
U.N.35	India, Malwa	0.70	12.21	0	0
U.N.26	Indo-China, Luang Prabang	0.57	11.58	0	0
U.N.7	Turkey, Merzifon, C.	2.15	20.80	0	0
U.N.38	Yugoslavia, No.2	0.53	18.28	0	0

*Calculated in terms of morphine.

30. The assay morphine, tested by the procedures described under paragraphs 20 to 22 inclusive, was found to be entirely free not only from non-phenolic alkaloids but also free from other phenolic alkaloids, so far as the method of dichloracetic acid is concerned. This shows again that preliminary removal of the non-phenolic alkaloids, before the dichloracetic-acid separation, is preferable.